ABSTRACT

A novel optical ruler based on ultrahigh-resolution colocalization of single fluorescent probes is described. Two unique families of fluorophores are used, namely energy-transfer fluorescent beads and semiconductor nanocrystal (NC) quantum dots, that can be excited by a single laser wavelength but emit at different wavelengths. A novel multicolor sample-scanning confocal microscope was constructed which allows one to image each fluorescent light emitter, free of chromatic aberrations, by scanning the sample with nanometer scale steps using a piezo-scanner. The resulting spots are accurately localized by fitting them to the known shape of the excitation point-spread-function of the microscope.